NF-κB activation induced by T cell receptor/CD28 costimulation is mediated by protein kinase C-θ

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Protein kinase C-θ (PKCθ) is a Ca^{2+}-independent member of the PKC family that is selectively expressed in skeletal muscle and T lymphocytes and plays an important role in T cell activation. However, the molecular basis for the important functions of PKCθ in T cells and the manner in which it becomes coupled to the T cell receptor-signaling machinery are unknown. We addressed the functional relationship between PKCθ and CD28 costimulation, which plays an essential role in T cell receptor-mediated IL-2 production. Here, we provide evidence that PKCθ is functionally coupled to CD28 costimulation by virtue of its selective ability to activate the CD28RE/activator protein-1 (AP-1) element in the IL-2 gene promoter. First, CD28 costimulation enhanced the membrane translocation and catalytic activation of PKCθ. Second, among several PKC isoforms, PKCθ was the only one capable of activating NF-κB or CD28RE/AP-1 reporters in T cells (but not in 293T cells). Third, wild-type PKCθ synergized with CD28/CD3 signals to activate CD28RE/AP-1. In addition, PKCθ selectively synergized with Tat to activate a CD28RE/AP-1 reporter. Fourth, CD3/CD28-induced CD28RE/AP-1 activation and NF-κB nuclear translocation were blocked by a selective PKCθ inhibitor. Last, PKCθ-mediated activation of the same reporter was inhibited by the proteasome inhibitor MG132 (which blocks IκB degradation) and was found to involve IκB-kinase β. These findings identify a unique PKCθ-mediated pathway for the costimulatory action of CD28, which involves activation of the IκB-kinase β/IκB/NF-κB-signaling cascade.

T cell activation induced by triggering of the antigen-specific T cell receptor (TCR)/CD3 complex in concert with costimulatory and adhesion receptors is a complex process that involves multiple enzymes, adapters, and other cellular proteins. Activation is initiated by stimulation of TCR-coupled protein tyrosine kinases of the Src and Syk families, which then phosphorylate various cellular substrates (1). These events are followed by the recruitment and assembly of membrane-signaling complexes that mediate different signal transduction pathways. These signals are relayed to the nucleus, where they induce a defined genetic program, of which the best characterized constituent is the activation of the IL-2 gene by coordinated binding of several transcription factors to the IL-2 gene promoter (2).

Members of the protein kinase C (PKC) family also play an important role in T cell activation (3). Although the contribution of individual PKC isoform to T cell activation is unknown, recent interest has focused on PKCθ, a Ca^{2+}-independent PKC isoform that is selectively expressed in T cells and in muscle (4–7). This is based on the findings that PKCθ specifically activates c-Jun N-terminal kinase (JNK) and activator protein-1 (AP-1) in T lymphocytes and synergizes with calcineurin to activate the IL-2 gene (8–10). Secondly, PKCθ selectively colocalizes with the T cell antigen receptor (TCR) to the core of the supramolecular activation complex formed in the contact region between antigen-specific T cells and antigen-presenting cells (11, 12). However, the molecular basis for the important functions of PKCθ in T cells and the manner in which it becomes coupled to the TCR-signaling machinery are unknown.

CD28 costimulation plays an essential role in TCR-mediated IL-2 production (13–15). The selective activation of JNK/AP-1 by PKCθ, the essential role of CD28 costimulation in JNK activation (16), and the localization of both PKCθ (11, 12) and, possibly, CD28 (17), to the central supramolecular activation complex prompted us to study potential functional interactions between CD28 and PKCθ. We show that PKCθ plays an important and selective role in CD28 costimulation by activating the IκB-kinase β (IKKβ)/IκB/NF-κB-signaling cascade.

Materials and Methods

Plasmids. The 4xRE/AP-luciferase reporter (18) was obtained from A. Weiss (University of California, San Francisco). The NF-κB- and AP-1-luciferase reporter plasmids were obtained from M. Karin (University of California, San Diego). The pEF4-LacZ reporter plasmid was obtained from Invitrogen. A Tat cDNA was generated by reverse transcription of RNA extracted from HIV-1/Lai-infected cells. The two exons of Tat (amino acids 1–86) were subcloned by reverse transcription–PCR into the *Eco*RI and *Xba*I sites of the pEF4/myc-His mammalian expression vector (Invitrogen). The stop codon was removed, and the insert was subcloned in-frame to the C-terminal c-Myc tag. IKKα and IKKβ were excised from the pEV3S and pcDNA3.1 vectors, respectively (obtained from W. Greene, Gladstone Institute, San Francisco), by digestion with *Kpn*I/*Nhe*I and *Xba*I/*Hin*dIII, respectively, blunted, and subcloned into the *Eco*RV site of the pEF4/myc-His vector. The IKKβ plasmid encodes a C-terminal Flag epitope derived from the original vector. The cDNAs encoding human wild-type or constitutively active mutants of human PKCθ and α, rat PKCε, or mouse PKCζ have been described (19). Xpress epitope-tagged versions of these PKCs were generated by using the pEF4/His mammalian expression vector (Invitrogen).

Cell Culture, Transfection, and Reporter Assays. Human leukemic Jurkat (E6.1) and 293T cells were cultured as described (20). Cells in a logarithmic growth phase were transfected with the indicated amounts of plasmid DNAs by electroporation as described (19, 20). Cells were stimulated for the indicated times with combinations of anti-CD3 and/or CD28 antibodies (PharMingen) or with tumor necrosis factor α (TNFα; Genzyme). In some experiments, the cells were treated with rottlerin or Gö6976 (both from Calbiochem) or MG132 (Biomol) as indicated. Transfected Jurkat cells were harvested after 24 h, washed twice with PBS, and lysed, and luciferase or β-galactosidase activities were determined as described (19). The results were expressed as arbitrary luciferase units normalized to β-galactosidase activity in the same cells. All experiments were performed at least twice with similar results.

Abbreviations: PKC, protein kinase C; TCR, T cell receptor; CD28RE, CD28 response element; AP-1, activator protein-1; TNF, tumor necrosis factor; IKK, IκB kinase; JNK, c-Jun N-terminal kinase.

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Immunoprecipitation and Western Blotting. These procedures were performed as described (19, 20). In brief, cells were lysed, and the supernatants obtained after centrifugation were incubated with optimal concentrations of primary antibodies, followed by addition of protein G-plus-Sepharose (Pharmacia). Washed immunoprecipitates were dissolved in Laemmli buffer, resolved by SDS/PAGE, and transferred to nitrocellulose membranes, which were blocked with 5% dry milk. The membranes were incubated with blocking buffer containing optimal concentrations of blotting antibodies, washed, and incubated with horseradish peroxidase-conjugated secondary anti-rabbit or -mouse IgG antibodies (Amersham). After washing, the blots were developed by using an enhanced chemiluminescence kit (Amersham Pharmacia). As control for protein loading, samples were also immunoblotted with an anti-actin mAb (ICN).

Kinase Assays. Endogenous PKCθ was immunoprecipitated by using a polyclonal antibody (Santa Cruz Biotechnology), and transfected IKKα or IKKβ were immunoprecipitated by using mAbs specific for the c-Myc (Santa Cruz Biotechnology) or Flag (Sigma) epitopes, respectively. Immunoprecipitates were resuspended in 20 μl of the respective kinase buffers (19, 21) containing 5 μCi of [γ-^{32}P]ATP and 1 μg of myelin basic protein or glutathione *S*-transferase-IκBα/1–62 as substrates for PKC or IKK, respectively. Where indicated, rottlerin or Gö6976 was added to the PKC kinase reactions. Reactions were incubated for 20–30 min at 30°C with gentle shaking, subjected to SDS/PAGE, transferred to nitrocellulose, and developed by autoradiography. [γ-^{32}P]ATP incorporation was determined by using a STORM 860 PhosphorImager (Molecular Dynamics). Nitrocellulose membranes were reprobed with the corresponding kinase- or tag-specific antibodies to determine expression levels of the immunoprecipitated kinases.

Subcellular Fractionation. To determine PKCθ redistribution, cells were fractionated into cytosolic or membrane fractions as described (22), and SDS/PAGE-resolved proteins were immunoblotted with a horseradish peroxidase-conjugated anti-PKCθ mAb (Transduction Laboratories, Lexington, KY). For NF-κB translocation, nuclear and cytoplasmic extracts were prepared and stored at −80°C as described (23). Extracts were resolved by SDS/PAGE, transferred to nitrocellulose, and immunoblotted with a polyclonal anti-RelA (p65) antibody (Santa Cruz Biotechnology).

Results

CD28 Costimulation Enhances the Translocation and Activity of PKCθ. T cell activation is associated with translocation of PKCθ to the membrane (7, 24) and, more specifically, to the T cell synapse (11, 12). To assess the contribution of CD28 costimulation to these events and to the activation of PKCθ, we compared the effects of anti-CD3 and/or CD28 antibody stimulation on the localization and activity of PKCθ in T cells. Costimulation of Jurkat T cells with both antibodies enhanced in parallel the translocation (Fig. 1*a*) and *in situ* catalytic activity (Fig. 1*b*) of PKCθ by comparison with either single stimulus. Both responses peaked at 1–10 min and declined after 30 min. These effects are mediated by a Vav/Rac pathway that acts selectively on PKCθ but not on other T cell-expressed PKC isoforms (20).

Selective Activation of NF-κB and CD28RE/AP-1 by PKCθ. CD28 is known to mediate its costimulatory function by activating the CD28RE/AP-1 element in the IL-2 gene promoter (18, 25–28). Therefore, we analyzed the role of PKCθ in activating this element. As shown in Fig. 2*a*, a constitutively active mutant (A/E) of PKCθ, but not α, ζ, or ε mutants, induced marked activation of the CD28RE/AP-1 reporter in transiently cotransfected T cells. As reported for anti-CD3/CD28 costimulation

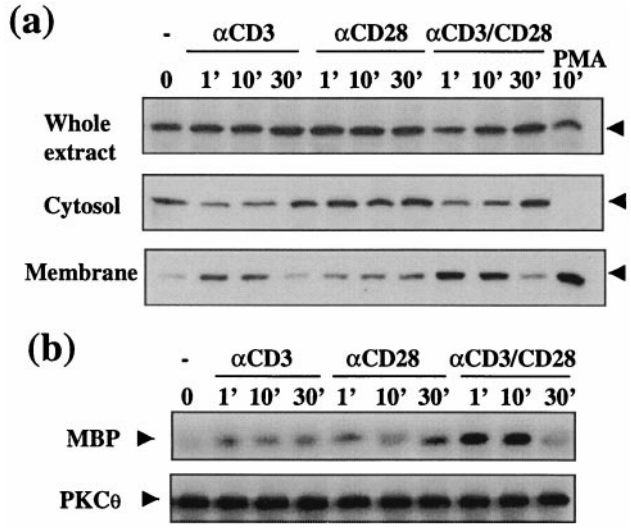

Fig. 1. CD28 costimulation enhances membrane translocation and *in situ* catalytic activity of PKCθ. (*a*) Jurkat T cells were stimulated with anti-CD3 and/or anti-CD28 antibodies (2 μg/ml each) for the indicated times. Whole extract, cytosol, and membrane fractions were prepared and resolved by SDS/PAGE, and the expression of PKCθ in each fraction was determined by Western blotting. PMA stimulation (100 ng/ml for 10 min) was used as a positive control for PKC translocation. The position of PKCθ is indicated by arrowheads. (*b*) Jurkat cells were stimulated as in *a*. Endogenous PKCθ was immunoprecipitated, and its enzymatic activity was determined (*Upper*). Kinase reactions were performed in the absence of lipid cofactors or PMA to reflect the *in situ* activity of PKCθ (11). SDS/PAGE resolved reaction products were analyzed by autoradiography (*Upper*). The membrane was immunoblotted with a PKCθ-specific antibody (*Lower*).

(18, 28), the effect of PKCθ required both NF-κB- and AP-1-binding sites because CD28RE/AP-1 reporter constructs in which either site was mutated were not activated by PKCθ (data not shown).

Because the CD28RE/AP-1 element contains binding sites for both AP-1 and NF-κB (18, 25–28), and we previously found that PKCθ is a selective AP-1 activator in T cells (8), we determined whether PKCθ can also activate an isolated NF-κB reporter. The PKCθ-A/E mutant induced strong activity of the NF-κB-Luc reporter, whereas other PKC isoforms induced very weak (α, ε) or no (ζ) activity (Fig. 2*b*). The PKCθ-induced NF-κB activity was not enhanced by additional stimulation with phorbol ester (data not shown), suggesting that PKCθ is the predominant, if not exclusive, mediator of NF-κB activation. Interestingly, the effect of PKCθ on NF-κB was cell-specific because PKCθ (and PKCε) stimulated low NF-κB activity in 293T cells, whereas PKCα displayed the highest activity in these cells (Fig. 2*c*). All PKC isoforms tested were properly overexpressed in the cells (Fig. 2 *Lower*) and, furthermore, were functional as indicated by their ability to stimulate the activity of a cotransfected ERK2 reporter (ref. 9 and data not shown).

Several experiments were carried out to further establish the functional coupling of PKCθ to the CD3/CD28 costimulation pathway. First, wild-type PKCθ synergized with anti-CD3 plus -CD28 antibodies to activate a CD28RE/AP-1 reporter (Fig. 3*a*). Second, we tested the ability of constitutively active PKC mutants to synergize with the HIV-1-derived protein, Tat. This was based on findings that HIV-1 infection or Tat overexpression synergizes with CD3/CD28 costimulation to superinduce the IL-2 and IL-8 genes; this effect is mediated by the action of Tat on the CD28RE (29, 30). Among four PKC isoforms tested, only PKCθ could synergize with cotransfected Tat to activate the

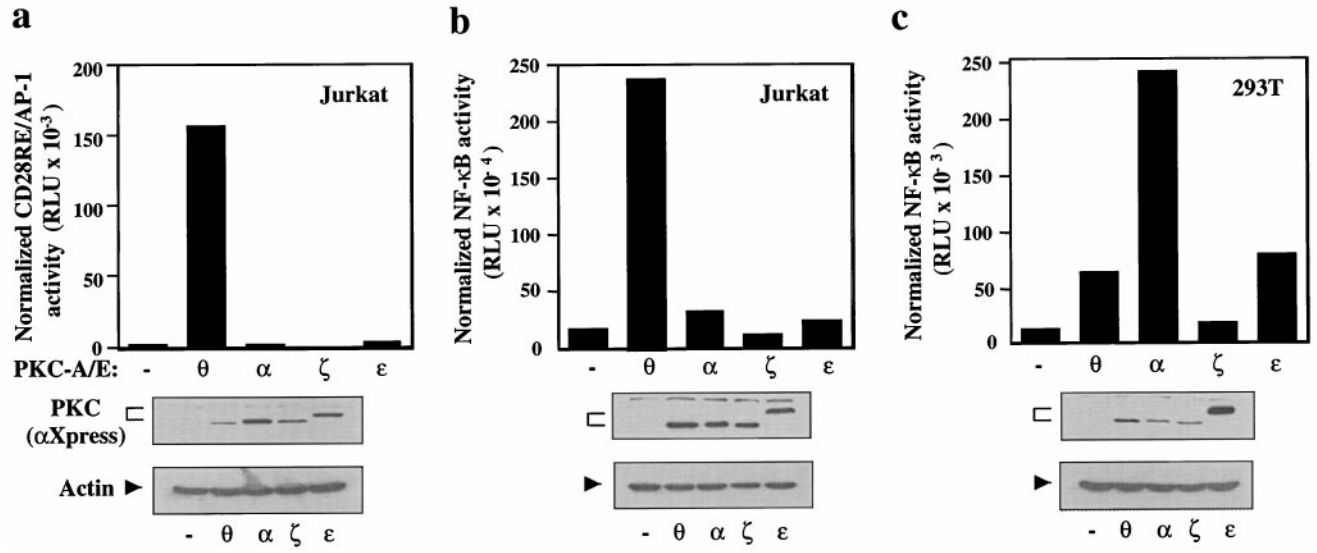

Fig. 2. PKCθ selectively activates NF-κB and the CD28RE/AP-1 element of the IL-2 promoter in a T cell-specific manner. The 1×10^7 Jurkat T cells (*a* and *b*) or 2×10^6 293T cells (*c*) were transfected with CD28RE/AP-1 (*a*) or NF-κB-Luc (*b* and *c*) reporters (5 μg each) in the presence of empty vector (pEF; −) or constitutively active (A/E) PKC-θ, -α, -ζ or -ε mutants (10 μg each). After 24 h, cells were lysed and luciferase activity was quantified. The expression level of the different PKCs was analyzed by Western blotting with an anti-Xpress antibody, and anti-actin immunoblotting was used as a control for protein loading (*Lower*).

CD28RE/AP-1 reporter (Fig. 3*b*). These results suggest that PKCθ is functionally coupled to CD3/CD28 costimulation.

Inhibition of CD28RE/AP-1 Activation by a Selective PKCθ Inhibitor. The importance of PKCθ in CD28RE and NF-κB activation was assessed by analyzing the effect of rottlerin, originally found to be a selective PKCδ inhibitor (31). We recently found that rottlerin also inhibits PKCθ function *in vitro* and in intact T cells and, furthermore, that these cells do not express PKCδ (19). Rottlerin inhibited the anti-CD3/CD28-stimulated activity of CD28RE/AP-1 by ≈80% (Fig. 4*a*), and essentially blocked the receptor-stimulated nuclear translocation of RelA (p65) (Fig. 4*b*), an NF-κB component that is known to bind to the CD28RE/AP-1 element (25). Rottlerin did not inhibit, however, NF-κB activity induced by TNFα (data not shown), indicating that CD3/CD28 and TNFα signals activate NF-κB via distinct pathways (21). The specificity of these inhibitory effects is evident from the finding that Gö6976, a PKC inhibitor selective for Ca^{2+}-dependent PKC isoforms (32), caused minimal inhibition of these functions. A control experiment confirmed that rottlerin was considerably more effective than Gö6976 in inhibiting CD3/CD28-induced PKCθ activity (Fig. 4*c*).

PKCθ-Mediated NF-κB and CD28RE/AP-1 Activation Involves IκB and IKK. Additional experiments were conducted to elucidate the pathway leading from PKCθ to NF-κB activation, and further establish the physiological relevance of PKCθ in these events. The selective proteasome inhibitor, MG132, which prevents IκB degradation (33), blocked in a dose-dependent manner the PKCθ-A/E-induced activation of NF-κB (Fig. 5*a*) and CD28RE/AP-1 (Fig. 5*b*), but not of AP-1 (Fig. 5*c*). Similar results were obtained with an IκB phosphorylation inhibitor, BAY 11–7082 (data not shown), indicating that IκB degradation is important.

Next, we determined the role of IKK in PKCθ-mediated CD28RE/AP-1 activation. Similar to anti-CD3/CD28 stimulation which has been reported to activate IKK (34, 35), constitutively active PKCθ also induced significant activation of IKKβ (but not IKKα) to an extent similar to that induced by TNFα or anti-CD3/CD28 stimulation (Fig. 5*d*). The biological relevance of this activation is indicated by the finding that a dominant-negative IKKβ mutant, which can inhibit CD3/CD28-induced activation of CD28RE/AP-1 (21, 34, 35), also inhibited activation of the same reporter induced by PKCθ; a dominant negative IKKα mutant was less active (Fig. 5*e*), possibly reflecting its potential contribution to the formation of an IKKα/IKKβ heterodimer. The ability of CD3/CD28 stimulation, but not PKCθ, to activate IKKα suggests that CD3/CD28 signals may activate IKKα via a PKCθ-independent pathway, but the physiological significance of IKKα in the context of CD28RE/AP-1 induction remains unclear. Both IKKs and PKCθ were properly overexpressed in the cells (Fig. 5*f*).

Discussion

Taken together, our results and very recent findings by others (36) identify a unique PKCθ-mediated pathway for the costimulatory action of CD28. This pathway involves activation of the IKK/IκB/NF-κB-signaling cascade, leading to stimulation of the combined CD28RE/AP-1 site in the IL-2 gene promoter. The importance of PKCθ in TCR-signaling pathway was previously inferred from several studies. First, among several T cell-expressed PKC isoforms, only PKCθ was capable of stimulating AP-1 and JNK activity and synergizing with calcineurin to activate the IL-2 gene (8–10). Second, the antigen-induced colocalization of PKCθ with the TCR to the contact region between T cells and antigen-presenting cells was not observed when T cells were activated under conditions that do not lead to proliferation (11, 12).

The inhibition of CD3/CD28-induced CD28RE/AP-1 activation by the PKC inhibitor, rottlerin (19, 20), suggests that the activation of NF-κB by PKCθ in T cells is a physiologically relevant event, consistent with the recently identified TCR-mediated activation defect in PKCθ-deficient T cells (D. Littman, personal communication). We also assessed the effects of dominant negative PKC mutants on receptor-mediated NF-κB or CD28RE/AP-1 activation. In addition to dominant negative PKCθ, which displayed the strongest inhibitory activity, dominant negative mutants of PKCα, -ε, or -ζ also inhibited reporter activation (data not shown). These results do not necessarily contradict the conclusion that only PKCθ (and not other PKCs)

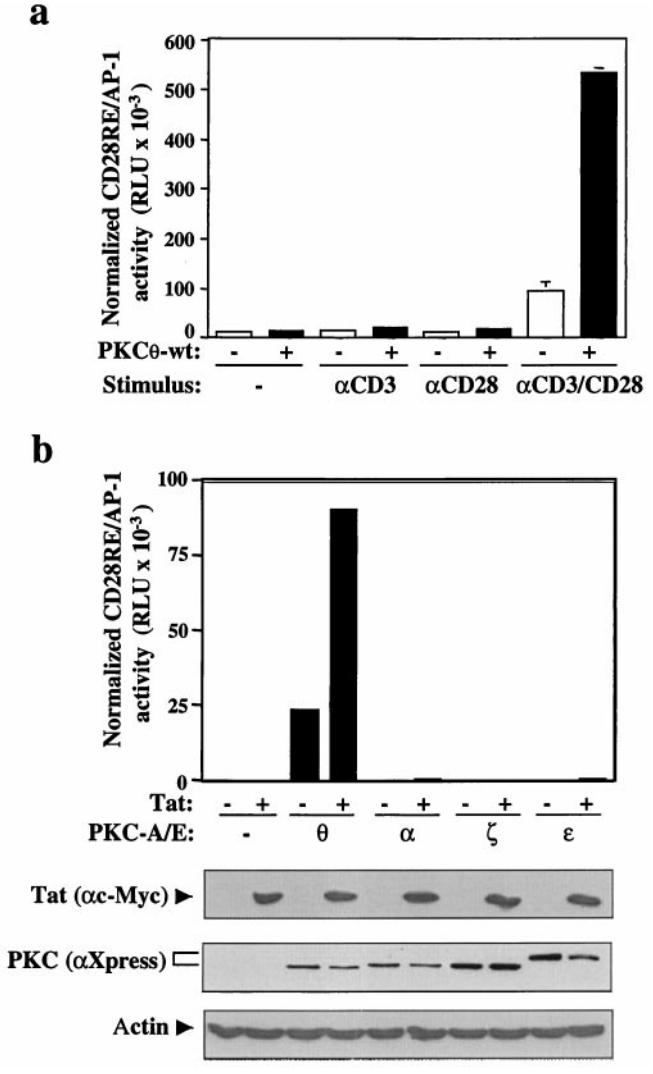

Fig. 3. PKCθ is functionally coupled to CD28 costimulation. (*a*) Jurkat T cells were transfected with an empty vector (−) or wild-type PKCθ (+) together with CD28RE/AP-1-Luc. After 20 h, cells were stimulated for an additional 6 h with CD3- and/or CD28-specific antibodies. Normalized luciferase activity in cell lysates was quantified as in Fig. 2. (*b*) CD28RE/AP-1-Luc activity in Jurkat T cells cotransfected with an empty vector (−) or with a c-Myc-tagged Tat plasmid together with constitutively active PKC-θ, -α, -ζ, or -ε mutants. The expression level of Tat or PKCθ was analyzed by Western blotting, and equal protein loading was confirmed by anti-actin immunoblotting (*Lower*).

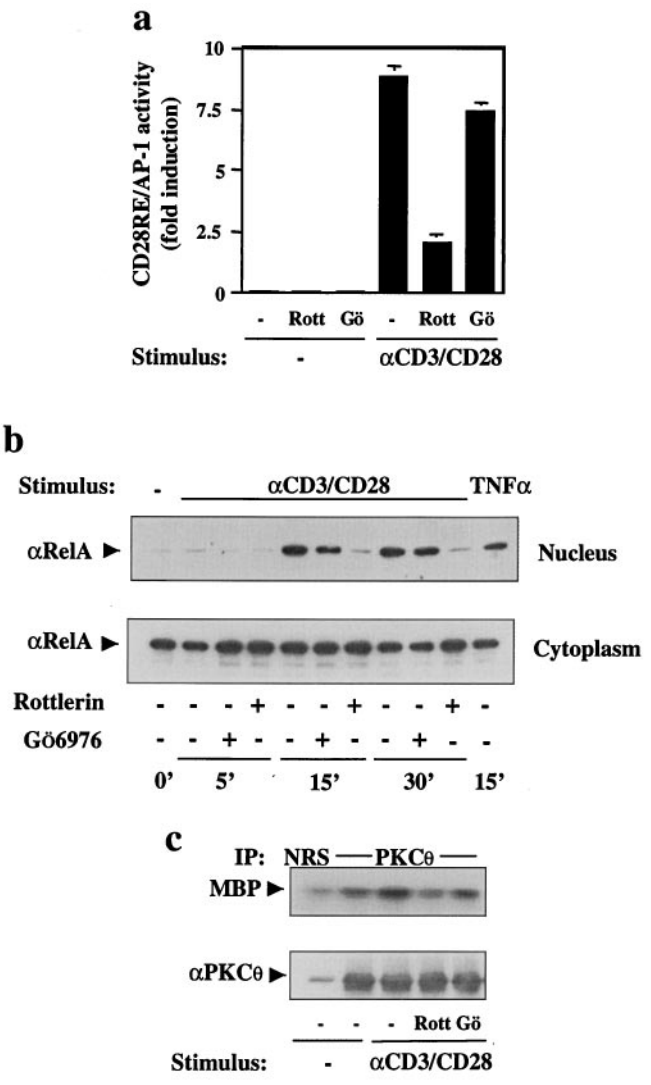

Fig. 4. Inhibition of PKCθ blocks CD28 costimulation. (*a*) Jurkat T cells were cotransfected with CD28RE/AP-1-Luc (10 μg) and β-galactosidase plasmid (2 μg) reporters. Cells were stimulated for 10 min (20 h later) with CD3- plus CD28-specific antibodies, in the presence or absence of rottlerin (Rott; 30 μM) or Gö6976 (Gö; 0.5 μM). Luciferase activity in cell lysates was determined. (*b*) Jurkat cells were incubated (15 min at 37°C) in the absence or presence of rottlerin or Gö6976 and then stimulated with anti-CD3/CD28 antibodies or with TNFα (10 ng/ml) for the indicated times. Nuclear and cytoplasmic extracts were prepared, and protein from each fraction (5 μg) was analyzed by Western blotting with an anti-RelA antibody. (*c*) Wild-type PKCθ-transfected Jurkat T cells were stimulated as in *a*. Cell lysates were immunoprecipitated (IP) with normal rabbit serum (NRS) or with an anti-PKCθ antibody, and the *in vitro* enzymatic activity of PKCθ was measured in the presence or absence of rottlerin or Gö6976 (*Upper*). The membrane was immunoblotted with a PKCθ-specific antibody (*Lower*).

functions as an intermediate in the TCR/CD28 pathway leading to NF-κB and CD28RE/AP-1 activation. Rather, we consider these results to be uninformative because cross-inhibition among similar dominant negative PKC mutants was recently reported and was inferred to reflect a common step in the phosphorylation and activation of various PKC isoforms (37).

The mechanism by which CD28 costimulation enhances TCR signals is not clear. CD28 ligation may generate unique biochemical signals (14) that cooperate with TCR signals to induce activation of the IL-2 gene and/or promote the clustering of glycolipid-enriched membrane lipid rafts (38) that form platforms for the TCR and other signaling elements essential for T cell activation (39). In general, it has been difficult to identify CD28-specific biochemical signals. Our results demonstrate that wild-type PKCθ did not cooperate with either anti-CD3 or -CD28 antibodies to enhance CD28RE/AP-1 activity (Fig. 3*a*). Rather, the effect of PKCθ was observed only when the cells were stimulated with a combination of both antibodies. Therefore, PKCθ overexpression does not replace either of the two receptor signals. Similarly, although anti-CD3 stimulation caused some membrane translocation and activation of PKCθ, which was enhanced by CD28 costimulation (Fig. 1), it is obvious that the effects of TCR/CD3 ligation alone on PKCθ are not sufficient to induce CD28RE/AP-1 activity (Fig. 3). Thus, PKCθ does not appear to be uniquely coupled to the TCR or CD28. Instead, our results favor the notion that CD28 induces costimulation by acting as a general amplifier of early TCR signals (38, 40).

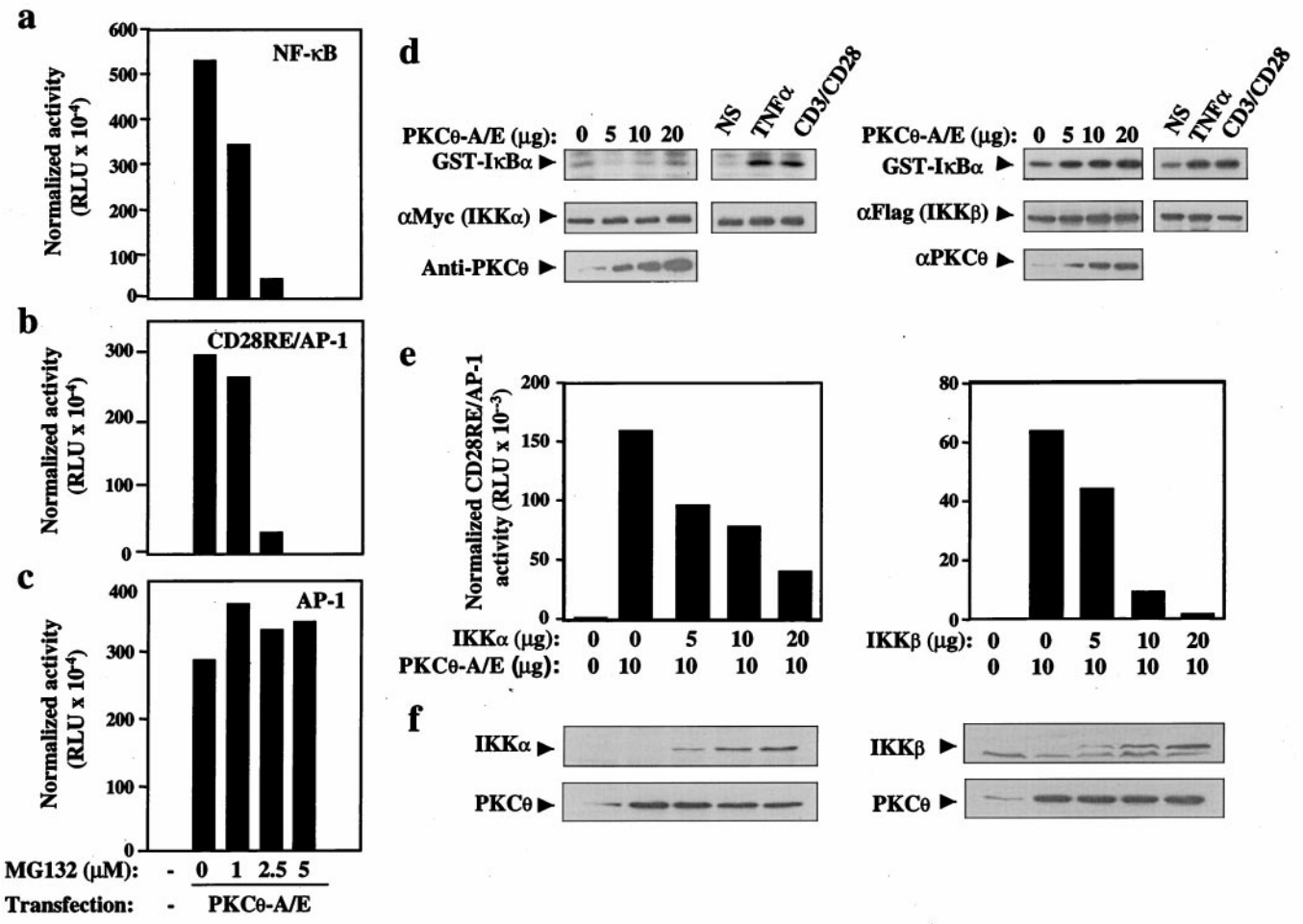

Fig. 5. NF-κB activation induced by PKCθ is mediated by IKKβ/IκBα. Jurkat cells were transfected with an empty vector (−) or constitutively active PKCθ (10 μg) together with NF-κB-Luc (*a*), CD28RE/AP-1-Luc (*b*), or AP-1-Luc (*c*) reporter constructs (5 μg each). The cells were cultured for 16 h with the indicated concentrations of MG132 and lysed, and luciferase activity was quantified. (*d*) Jurkat cells were transfected with wild-type IKKα (5 μg) or IKKβ (2 μg) together with an empty vector (0) or increasing amounts of constitutively active PKCθ. Twenty-four hours later, the cells were stimulated with anti-CD3/CD28 antibodies or with TNFα for 10 min. Immunoprecipitated IKKα or IKKβ were subjected to an *in vitro* kinase assay. Phosphorylated glutathione *S*-transferase-IκBα/1–62 was detected by autoradiography (*Top*). The same membrane was immunoblotted with anti-c-Myc or anti-Flag antibodies (*Middle*). (*Bottom*) The expression level of PKCθ-A/E. (*e*) Jurkat cells were transfected with an empty vector or with constitutively active PKCθ (10 μg) in the absence or presence of increasing amounts of kinase inactive IKKα or IKKβ mutants, together with a CD28RE/AP-1-Luc reporter (5 μg). After 24 h, cells were lysed and normalized luciferase activity was determined. (*f*) The expression level of the transfected IKKs or PKCθ was assessed by Western blotting using c-Myc- (IKKα), Flag- (IKKβ), or PKCθ-specific antibodies.

The precise mechanism that leads from PKCθ to NF-κB activation, and the nature of the intermediates between PKCθ and the IKK/IkB cascade, remain to be determined. In this regard, one candidate for mediating the effects of PKCθ is the Akt (PKB) kinase, which was recently found to activate NF-κB and CD28RE/AP-1 in T cells (41). However, the effects of PKCθ and Akt differ from each other. First, although constitutively active PKCθ activated NF-κB and CD28RE/AP-1 in unstimulated cells, activation mediated by wild-type Akt or even by active, membrane-targeted Akt required stimulation with anti-TCR antibody plus phorbol ester. Second, unlike PKCθ, Akt did not activate NFAT or AP-1. Finally, Akt, but not PKCθ, was capable of activating IKKα (41). These differences are more consistent with the notion that PKCθ and Akt act in separate pathways.

Three distinct MAP3 kinases were found to activate NF-κB and CD28RE/AP-1 in T cells, i.e., MEKK1, NF-κB-inducing kinase (NIK), and Cot (21, 35). Moreover, NIK has tentatively been placed downstream of Cot in the TCR/CD28-signaling pathway leading to NF-κB activation (21). Both NIK and MEKK1 also activate the IKK cascade in other cell types (42–45). The findings that PKCθ selectively activates both the JNK/AP-1 pathway (8–10) and the IKK/IκB/NF-κB pathway (as shown herein), and that MEKK1 mediates cross-talk between the JNK and IKK cascades (35), raises the possibility that MEKK1 is a target for PKCθ in T cells. However, so far we have not been able to block the PKCθ-mediated activation of CD28RE/AP-1 in T cells by coexpressing a dominant negative MEKK1 mutant; in contrast, dominant negative Cot was capable of inhibiting this activation (data not shown). This finding raises the possibility that Cot is a PKCθ target in T cells. This notion is consistent with the ability of Cot to up-regulate expression of the IL-2 gene and to simultaneously activate several kinases and transcription factors (21, 46–50) that are also induced by PKCθ, i.e., JNK, ERK, NFAT, and NF-κB. Experiments to further address a potential functional link between PKCθ and Cot are in progress.

The activation of NF-κB by PKCθ, and its previously reported selective activation of AP-1 in T cells (8), most likely accounts for the highly selective ability of PKCθ to activate the CD28RE/

AP-1 element in the IL-2 gene promoter, which is known to bind components of both NF-κB and AP-1 transcription factor complexes (18, 25–28). Our results also suggest that the selective action of PKCθ, in conjunction with calcineurin, in activating the IL-2 gene (9, 10, 51) may reflect its activation of the CD28RE/AP-1 element in the corresponding promoter. Finally, the pathway revealed by our findings provides a molecular basis for the defective TCR-mediated activation of PKCθ-deficient peripheral T lymphocytes (D. Littman, personal communication). Therefore, selective pharmacological strategies designed to modulate the recruitment of PKCθ to the supramolecular activation complex, and/or its catalytic activity, may prove therapeutically useful for modulating T cell costimulatory signals in immunological diseases.

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